
EVIDENCE FOR MOTH AND BUTTERFLY POLLINATION IN *GLADIOLUS* (IRIDACEAE–CROCOIDEAE)¹

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ABSTRACT

Pollination strategies of *Gladiolus*, one of the largest genera of the monocot family Iridaceae, are unusually diverse, and include various bee species, foraging either for nectar or for pollen, passerine birds, long-proboscid flies foraging for nectar, hopliine beetles that use the flowers primarily as sites for assembly, and Lepidoptera. Pollination by insects of this order comprises two entirely different sets of pollinators, night-flying moths (Noctuidae or Sphingidae) and butterflies (evidently only one species of Satyridae). These lepidopteran-pollinated flowers have quite different floral adaptations and both types are specialist systems, although moth flowers may be pollinated by a range of different moths. In *Gladiolus* moth-pollinated flowers are usually large, long-tubed and pale-colored, or mottled dull purple to brown, are usually richly scented, often open fully only at night, and produce relatively concentrated nectar that is sucrose-rich. Butterfly flowers, in contrast, are fully open during the day, close partially or completely at night, are often bright crimson to scarlet, usually with prominent white splashes on the lower tepals, but are also large, have a long tube, and produce quantities of relatively dilute nectar, either sucrose-rich or hexose-rich. Comparing the pollination systems of related species, we infer that night-flying moth pollination arose 6 times in the genus, whereas butterfly pollination arose 3 times in the 165 species of southern Africa, and that the two lepidopteran pollination systems in *Gladiolus* arose quite independently of one another.

Key words: butterflies, floral ecology, *Gladiolus*, Iridaceae, moths, nectar, pollination systems.

Pollination in the approximately 35 genera of the Iridaceae of sub-Saharan Africa, where over 1000 species of the family occur, is remarkably diverse. Across the continent pollination systems include four orders of insects (Coleoptera, Diptera, Hymenoptera, Lepidoptera) and passerine birds, as well as facultative, and possibly obligate autogamy. Pollination by insects of the orders Diptera, Hymenoptera, and Lepidoptera is itself diverse, involving a range of families of each order and different sets of floral adaptations and associated rewards to pollinators. Here we present evidence for two different pollination strategies utilizing Lepidoptera in the large, predominantly African genus *Gladiolus*. Elsewhere we have documented passive pollination in the genus by large-bodied apid-anthophorine bees (Goldblatt et al., 1998a), hopliine beetles (Goldblatt et al., 1998b), long-proboscid flies in the families Nemestrinidae and Tabanidae with elongate mouth parts exceeding 15 mm (Goldblatt & Manning, 1999, 2000), and an active pollination system involving pollen-collecting female bees

(Goldblatt et al., 1998a). We have also presented evidence for passerine bird pollination in *Gladiolus* and reviewed pollination systems for the entire genus (Goldblatt et al., 1999, 2001). By comparing and contrasting the evidence for moth versus butterfly pollination in *Gladiolus*, we show just how different the two systems are that use lepidopteran pollen vectors.

Pollination by large butterflies, so far in *Gladiolus* known to involve only the satyrid, *Aeropetes tulbaghia*, has already been demonstrated for two species of *Gladiolus* and was inferred for several more because of the distinctive floral features present in species pollinated by this butterfly (Johnson & Bond, 1994). These features include a large, unscented flower, bright red perianth often with white splashes on the lower tepals, a narrow but fairly long perianth tube, and the presence of large quantities of nectar (Johnson & Bond, 1994; Goldblatt & Manning, 1998). Night-flying moth pollination has been inferred for nine species of *Gladiolus* based on the presence of features commonly asso-

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ciated with moth pollination, including a pale-colored perianth, presence of fairly concentrated nectar, and a strong, often distinctive scent with a clove component, and in some species floral odor produced only at night (Goldblatt & Manning, 1998). Field observations and investigation of floral features allow us to expand our understanding of these pollination systems. Moreover, by using information about phylogenetic relationships in the genus and comparing the pollination systems of related species, we can infer the number of times that each system evolved within the genus and suggest ancestral species, thus developing hypotheses about the evolution of the floral traits associated with pollination by different Lepidoptera.

MATERIALS AND METHODS

SPECIES EXAMINED

Observations on the floral and pollination biology of *Gladiolus* were made during the years 1993 to 1999 in the field in southern Africa and in living collections at the Missouri Botanical Garden, St. Louis, and Kirstenbosch Botanic Gardens, Cape Town. Together with our research on the systematics of the genus (Goldblatt & Manning, 1998; Manning et al., 1999), we have identified 20 species that have flowers likely to be pollinated by Lepidoptera from a total of 165 species of *Gladiolus* that occur south of the Limpopo–Cunene River axis. At least an additional 15 species of tropical Africa (Goldblatt, 1996) have similar flowers and may be assumed to be pollinated by Lepidoptera. Southern African species fall in five of the seven sections recognized in the genus by Goldblatt and Manning (1998: sects. *Blandus*, *Hebea*, *Homoglossum*, *Linearifolius*, and *Ophiolyza*). Species in two additional sections of the genus, sections *Acidanthera* and *Decoratus*, in tropical Africa also exhibit features consistent with pollination by Lepidoptera.

Candidates for Lepidopteran pollination were recognized by two separate suites of characters. The first is the classic syndrome for moth pollination (Faegri & van der Pijl, 1979) in which pale flower color and production of a strong, rich odor are associated with some features only exhibited at night. In *Gladiolus* such flowers are usually large, have an elongate floral tube, shortly exerted unilateral stamens, and the strong floral odor is often produced or intensified at night. Pigmentation may be cream or whitish, but several species have darkly mottled to nearly uniformly dark brown perianths. A second syndrome of characters, described in detail by Johnson and Bond (1994), combines bright red floral pigmentation, a large flag-like presenta-

tion, absence of floral odor, and often lower tepals marked with white splashes (we use the term tepal for perianth lobe here).

SEASONALITY, FLORAL PHENOLOGY, LONGEVITY, AND FLORAL PRESENTATION

Direct phenological observations are presented on 18 southern African species of *Gladiolus* made during the years 1993 to 1998 in the field (Table 1) and in living collections at Kirstenbosch Botanic Gardens, Cape Town. Observations include mode and timing of anthesis (i.e., opening of individual buds), anther dehiscence, expansion of stigmatic lobes, followed by withering of the perianth. Data on seasonality are taken from Goldblatt and Manning (1998). Compatibility relationships were not examined for the study. Plant vouchers (Table 1) are deposited at the Missouri Botanical Garden Herbarium, St. Louis (MO), and/or the Compton Herbarium, Cape Town (NBG).

NECTAR ANALYSIS

Nectar volume measurements were taken from unbagged flowers in the field, reflecting both rates of secretion and depletion, and from plants maintained in the laboratory, and not visited by insects (effectively representing bagged flowers). Experience with Iridaceae has shown that nectar characteristics gradually change in species retained in water for periods greater than 24 hours, the nectar usually becoming more diluted (Goldblatt et al., 1995). Nectar from flowers on cut stems maintained in water in the laboratory was therefore sampled within 18 hours. To collect nectar whole flowers were picked and nectar was withdrawn from the base of the perianth tube with 3 μ l capillary tubes after separating the ovary from the perianth base (perianth tubes are too elongate and curved to allow nectar removal directly via the mouth of the tube with a capillary tube). Detaching the perianth from the top of the ovary causes minimal damage, and contamination of nectar by fluid from broken tissue is not significant given the large volumes of nectar produced by flowers of species under investigation. The percentage of sucrose equivalents in fresh nectar was measured in the field or laboratory on a Bellingham and Stanley hand-held refractometer (0–50%) from five or more individuals per population, unless fewer individuals were available. Additional nectar samples were dried on Whatman filter paper no. 1 and analyzed by B.-E. van Wyk, Rand Afrikaans University, Johannesburg, using HPLC sugar analysis.

Table 1. Study sites and voucher information for species studied. Vouchers are housed at MO (Goldblatt) or at NBG (other collectors). All study sites are in South Africa.

Species	Study site and voucher
GLADIOLUS SECTION BLANDUS	
series <i>Blandus</i>	
<i>G. cardinalis</i> Curt.	W Cape, near Bain's Kloof, Jan., <i>Goldblatt & Manning s.n.</i> no voucher
<i>G. carmineus</i> C. H. Wright	W Cape, cliffs at Hermanus, Feb., <i>Goldblatt 11292</i>
<i>G. insolens</i> Goldblatt & J. C. Manning	W Cape, Piketberg, Jan., <i>Goldblatt & Manning 10166</i>
<i>G. stefaniae</i> Oberm.	W Cape, Potberg, Mar., <i>Goldblatt & Manning 10176</i>
<i>G. sempervirens</i> G. J. Lewis	E Cape, Tsitsikamma Mts., May, <i>Manning s.n.</i> no voucher
GLADIOLUS SECTION LINEARIFOLIUS	
series <i>Linearifolius</i>	
<i>G. emiliae</i> L. Bolus	W Cape, near Swellendam, Mar., <i>Manning 1057</i>
	W Cape, near Riviersonerend, Mar., <i>Stayner s.n.</i>
<i>G. guthriei</i> F. Bolus	W Cape, Bain's Kloof, May, <i>Manning 1080</i>
<i>G. nerineoides</i> G. J. Lewis	W Cape, Helderberg Reserve, Somerset West, Jan., <i>Runnals 463</i>
	W Cape, Jonkershoek Mts., Jan., <i>Esterhuysen 32847</i>
GLADIOLUS SECTION HEBEA	
series <i>Permeabilis</i>	
<i>G. robertsoniae</i> F. Bolus	Mpumalanga, near Morgenzon, Oct., <i>Goldblatt & Manning 10071</i>
GLADIOLUS SECTION HOMOGLOSSUM	
series <i>Gracilis</i>	
<i>G. albens</i> Goldblatt & J. C. Manning	E Cape, near Grahamstown, Mar., <i>Dold & Weeks s.n.</i>
<i>G. maculatus</i> Sweet	W Cape, Simonstown, June, <i>Manning s.n.</i> no voucher
	Devil's Peak, May, <i>Manning s.n.</i> no voucher
<i>G. recurvus</i> L.	W Cape, Helderberg Reserve, Nov., <i>Runnals 513</i>
series <i>Tristis</i>	
<i>G. hyalinus</i> Jacq.	W Cape, Gydo Pass, Sep., <i>Goldblatt & Manning 9743A</i>
	W Cape, Lion's Head, Aug., <i>Barker 3861</i>
<i>G. liliaceus</i> Houtt.	W Cape, Fairfield, Caledon, Sep., <i>Nänni s.n.</i> no voucher
<i>G. longicollis</i> Baker	Mpumalanga, Long Tom Pass, Feb., <i>Goldblatt & Manning 9822</i>
<i>G. tristis</i> L.	W Cape, near Bredasdorp, Aug., <i>Barker 2844</i>
GLADIOLUS SECTION OPHIOLYZA	
series <i>Oppositiflorus</i>	
<i>G. cruentus</i> T. Moore	KwaZulu-Natal, Hillcrest, Jan., <i>Goldblatt & Manning 9854</i>
<i>G. saundersii</i> J. D. Hook.	E Cape, Naude's Nek, Feb., <i>Goldblatt & Manning 9550</i>

Additional species with similar flowers, assumed to be adapted for pollination by *Aeropetes* or night-flying moths, include *G. acuminatus* F. Bolus (putatively moths) and *G. stokoei* G. J. Lewis (putatively butterflies), but we have no field observations of the floral ecology of these species. We also lack observations of insect visitors for *G. albens*, *G. carmineus*, *G. cruentus*, *G. hyalinus*, *G. insolens*, and *G. robertsoniae*, but those species are included above because we have nectar and other data from study populations.

FRAGRANCE

Floral scent was noted with the human nose in the field and in cultivated plants. Presence of scents too weak to be discerned in the open air was recorded after individual flowers were picked and placed in clean, lidded glass jars and stored in a warm place. The contents of each jar was sniffed after a minimum of 60 minutes (Buchmann, 1983). The site of scent production was examined by immersing flowers in aqueous neutral red stain. Scent chemistry was examined by R. Kaiser, Givaudan-Roure Research Ltd., Switzerland, by gas chromatography using a DB-Wax Capillary column (Kaiser, 1993). Scents were captured in capsules through

which air was drawn by a vacuum pump from a small, lidded chamber containing open flowers.

POLLINATION MECHANISMS AND POLLEN LOAD ANALYSES

Observations of insects on *Gladiolus* flowers involved at least 5 hours total per species and in some cases up to 20 hours total per species. For species inferred to be pollinated by moths, an additional 4 hours of observation was made per species during daylight hours to determine whether diurnal visits by insects might also occur. Observations included mode of foraging and whether insects contacted anthers and stigmas during vis-

its to flowers. Study populations always included at least 20 individuals at evidently undisturbed field sites. Insects observed to probe the floral tube or to brush the anthers or stigmas were netted when possible and, in the case of moths, then immobilized in a jar using ethyl acetate fumes. To prevent contamination of the body of an insect with pollen carried by another in the same jar, the bodies of insect specimens were isolated from each other by wrapping them in tissue prior to pinning. Individual butterflies were netted for measuring and observation of sites of pollen deposition and then released. Body length and proboscis length of insects was recorded from captured specimens. Body length was measured from the base of the labrum to the tip of the abdomen. Mouth-part length was measured from the base of the labrum to the tip of the proboscis. Night-flying moths are not easily captured simply because darkness makes them difficult to locate. Use of flashlights covered with translucent red cellophane paper for illumination significantly assisted observation and netting of moths. Captured moths were identified by Douglas Kroon (Sasolburg, South Africa), and both moth and butterfly voucher specimens were deposited with the South African Museum, Cape Town.

Identification of pollen on insect bodies was done by gently removing grains from the body surface with a dissecting needle. The residue from needle probes was collected on glass slides and mounted in 1–2 drops of Calberla's fluid (Ogden et al., 1974). Pollen grains were identified microscopically by comparison with reference to pollen grain preparations made from plants flowering at study sites. *Gladiolus* pollen grains are recognized by their large size, monosulcate aperture with prominent 2-banded operculum, and perforate-scabrate exine (Goldblatt et al., 1991).

RESULTS

SEASONALITY, FLORAL PHENOLOGY AND LONGEVITY, AND FLORAL PRESENTATION (TABLE 2)

Flowering times in the *Gladiolus* species of southern Africa that are pollinated by night-flying moths show no obvious association with their geographic ranges in the summer- or winter-rainfall zones of southern Africa (Goldblatt & Manning, 1998). Flowering of the flora peaks in late spring (September to November) in the winter-rainfall zone but in summer and early autumn (December to March) in the summer-rainfall zone. This coincides with the middle or end of the period of optimal plant growth, during or soon after the main rainy periods. In the summer-rainfall zone, *G. longicollis*

flowers early in the season, mostly October and November, sometimes producing a second flowering flush in December–January. The only other species of the region showing adaptation for moth pollination blooms in spring before the first rainfall of the wet season. This is out of phase with the main flowering period for *Gladiolus* in the summer-rainfall zone (Goldblatt & Manning, 1998).

In the winter-rainfall zone, *Gladiolus hyalinus*, *G. liliaceus*, and *G. tristis* follow the main flowering pattern for the region, but *G. albens* and *G. maculatus* flower early in the season, in autumn or winter, and *G. recurvus* flowers in late winter or early spring. Flowering out of phase with the flowering peak is also characteristic of the two moth pollinated species of section *Linearifolius*, *G. emiliae* and *G. guthriei*, which flower mainly in April and May, although vegetative growth in these two species is delayed until the winter and spring when leaves are produced.

Flowering of butterfly-pollinated species of *Gladiolus* is always from late December to April, exceptionally May, and this is when the only butterfly so far recorded on *Gladiolus* species, *Aeropetes*, is on the wing (Johnson & Bond, 1994). This means that for the butterfly-pollinated species of the winter-rainfall zone, flowering occurs several months out of phase with the main growth and flowering peak for the region. As a consequence these species must also have special ecological adaptations to support the unusual flowering patterns. These may be a specialized habitat, such as cliffs, stream banks, or waterfalls (*G. cardinalis*, *G. insolens*, *G. sempervirens*), or a growth pattern in which leaf production occurs when conditions are suitable, later in the season (*G. carmineus*, *G. stefaniae*), or both (*G. nerineoides*, *G. stokoei*).

Population density appears to be moderately diffuse, and plants form extended populations with flowering individuals standing 1–3 m apart. Species of specialized habitats such as *Gladiolus tristis* (moist to marshy sites), *G. cardinalis* (waterfalls), *G. carmineus* (coastal sandstone cliffs), *G. sempervirens* (seeps and wet forest margins) may be locally common and grow in dense stands.

The pattern of flower buds opening on an inflorescence is acropetal. In all species, a mature bud expands in the early to mid morning, and the open flower typically lasts four days in *Gladiolus* species (Goldblatt et al., 1998a; Goldblatt & Manning, 1999). In moth-pollinated *Gladiolus* species flowering lasts longer. Flowers of a cultivated sample of *G. tristis* lasted five or six days, and flowers of *G. recurvus* lasted five to nine days. Flowers usually open one to two days apart, and hence there are

Table 2. Floral and phenological data for southern African *Gladiolus* species with flowers adapted for pollination by Lepidoptera. Species are arranged taxonomically according to Goldblatt and Manning (1998). Perianth tube length was recorded at study sites and may not represent the range for the species. An asterisk (*) indicates species flowering out of phase with the peak flowering time for the rainfall zone.

Species	Flower color	Perianth tube (mm)	Scent	Main flowering time	Rainfall zone
GLADIOLUS SECTION BLANDUS					
series Blandus					
<i>G. cardinalis</i>	red, white streaks on lower tepals	32–40	none	late Dec.–Feb.	winter*
<i>G. carmineus</i>	crimson-pink, white streaks on lower tepals	30–35	none	Feb.–Mar.	winter*
<i>G. insolens</i>	scarlet-red	ca. 38	none	Jan.–Feb.	winter*
<i>G. stefaniae</i>	red, white streaks on lower tepals	35–45	none	Mar.–Apr.	winter*
<i>G. sempervirens</i>	red, white splashes on lower tepals	25–42	none	Mar.–May	winter*
GLADIOLUS SECTION LINEARIFOLIUS					
series Linearifolius					
<i>G. emiliae</i>	densely brown speckled	32–45	strong fruity	Mar.–Apr.	winter*
<i>G. guthriei</i>	dull purple-brown	20–27	sweet-clove	Mar.–June	winter*
<i>G. nerineoides</i>	scarlet	25–31	none	Jan.–Mar.	winter*
<i>G. stokoei</i>	carmine-red	30–35	none	Mar.	winter*
GLADIOLUS SECTION HEBEA					
series Permeabilis					
<i>G. acuminatus</i>	cream	16–22	sweet-floral	Aug.–Sep.	winter
<i>G. robertsoniae</i>	white	28–44	sweet-clove	Sep.–Oct.	summer*
GLADIOLUS SECTION HOMOGLOSSUM					
series Gracilis					
<i>G. albens</i>	white-cream	45–60	acrid-metallic	Mar.–May	winter*
<i>G. maculatus</i>	cream, heavily speckled brown to dull purple	23–35	sweet-floral	May–July	winter*
<i>G. recurvus</i>	cream to pale pink	27–36	sweet-clove	July–Sep.	winter
series Tristis					
<i>G. hyalinus</i>	cream speckled brown, purple or green	25–26	usually odorless or sweet-clove	Sep.–Nov.	winter
<i>G. liliaceus</i>	beige, buff or pale orange, light mauve at night	40–45	strong sweet-clove at night	Aug.–Nov.	winter
<i>G. longicollis</i> subsp. <i>longicollis</i>	cream, lightly speckled	100–110	strong sweet-clove at night	Sep.–Nov.	summer*
<i>platypetalus</i>	white to cream	85–110	strong sweet-clove at night	Oct.–Dec.	summer*
<i>G. tristis</i>	white to cream	40–60	strong sweet clove at night	Sep.–Nov.	winter
GLADIOLUS SECTION OPHIOLYZA					
<i>G. cruentus</i>	red, white on lower tepals	ca. 28	none	Jan.–Feb.	summer
<i>G. saundersii</i>	red, white on lower tepals	33–37	none	Feb.–Mar.	summer

often two or more flowers open at any time on an inflorescence of three or more flowers. Flowers of moth-pollinated species are usually partly closed or at least the tepals are flaccid during the day, and open fully at sunset when the tepals become firm and fully extended. In species of *Gladiolus* series *Tristis* of section *Homoglossum* scent is released at the same time, although a faint odor may be detected during the day.

The reverse pattern holds for butterfly-pollinated species. At sunset, the tepals of most species partly or fully close, the tepals then loosely enclosing the exerted anthers and stigmas. During the day the tepals become fully expanded again.

Flowers of many *Gladiolus* species have been found to exhibit mechanical protandry (Scott Elliot, 1891; Goldblatt et al., 1998a; Goldblatt & Manning, 1999), and the species studied here conform

to this pattern. The anthers dehisce longitudinally one to four hours after the tepals first unfold. This depends to some extent on ambient temperature and humidity, and anthers dehisce later in wet, cool conditions. Pollen grains are clumped together and pollen remains in the anther thecae until removed by an insect. The three style branches, the distal adaxial surfaces of which comprise the stigmas, are loosely held together for the first three (to five) days that the flower is open and lie laxly over on the dorsal surface of the anthers. On the last day of anthesis, the style elongates and the style branches diverge, arching outward beyond the anthers. At the same time, the conduplicate margins of the distal half of each style branch unfold, exposing the moist, sticky stigmatic surfaces of the now spatulate style branches. Only then are the stigmas of a flower accessible to pollen deposition, and pollen adheres to these areas following hand-pollination.

Thus of the four to six days that a flower is open, it typically has three to five days in an exclusively male phase during which time pollen is usually removed from the anthers by insects. Anthers can be seen with the naked eye to lack pollen after three or four days if flowers were actively visited. By the time the stigma lobes unfold the flower is then in an exclusively female phase that lasts for the final one (or two) days that a flower is open. Mechanical self-pollination cannot readily occur, even if pollen remains in the anthers by the time the receptive stigmatic areas are exposed because of the spatial separation of the pollen-bearing anthers and the stigmatic surfaces. *Gladiolus carmineus*, which we include here as possibly pollinated by large butterflies although we lack pollinator observations, is an exception. The style divides opposite the base of the anthers and the style branches are tangled in the dehiscent anthers; thus, selfing could easily occur if there were no incompatibility system (unknown at present). At the *G. carmineus* study site pollen had not been removed from the anthers, and the stigmatic surfaces become dusted with pollen from anthers of the same flower.

Species of *Gladiolus* are medium-sized, corm-bearing geophytes, typically 45–120 cm high (Fig. 1A–F). Species pollinated by Lepidoptera typically produce a single, unbranched flowering stem annually. Flowering in a population is synchronous and lasts two to four weeks, and inflorescences are typically secund spikes with the flowers facing to the side and with the floral tube in an ascending position. In *G. nerineoides* the flowers are crowded at the apex of the flowering stem in more or less spiral arrangement and it is the entire inflores-

cence, rather than individual flowers, that makes for a conspicuous display (Fig. 1B).

Flowers pollinated by moths are moderate in size to large, depending on sectional affinity (Fig. 1C–F). In species of *Gladiolus* sect. *Homoglossum* the perianth tube is mostly 23–60 mm long, but exceptionally 85–110 mm in *G. longicollis* subsp. *platyptalus*. The dorsal tepal, usually slightly larger than the other five tepals, is 32–45 mm long, thus shorter to about as long as the tube (Table 2). In *Gladiolus* sects. *Hebea* and *Linearifolius* flowers are somewhat smaller, and *G. acuminatus* has a floral tube 16–22 mm long and tepals 15–21 mm long. Irrespective of sectional affinity, the perianth tube is more or less cylindric but slightly wider toward the apex, with the slender lower portion 1.5–2 mm in diameter, and fairly straight or gently curved. Flowers are zygomorphic and the slightly larger dorsal tepal is typically inclined while the upper lateral tepals are spread outward. The lower tepals, usually slightly smaller than the upper three, are held loosely together and directed forward. The style and stamens are unilateral and arch to lie close to and just beneath the dorsal tepal and are thus contained within the enclosed space formed by the ascending tepals. The filaments are shortly exerted from the tube, or in *G. longicollis* not or barely exerted, and the anthers lie parallel to one another with the lines of dehiscence facing toward the center of the flower and the lower tepals. In many moth-pollinated *Gladiolus* flowers the tepals are somewhat attenuate with the tips recurved, a feature most exaggerated in *G. acuminatus* and *G. recurvus*.

Moth-pollinated flowers are either shades of white to cream or are lightly to densely mottled with dull purple to brown (Table 2; Fig. 1C–F). Each of the three lower tepals may have a weakly contrasting, darker median band, often collectively referred to as a nectar guide (Goldblatt & Manning, 1998). The bases of the tepals and the distal part of the tube together form a wide throat leading to the narrow, proximal part of the tube. In the sense of Faegri and van der Pijl (1979), these are gullet flowers but often with a particularly elongated floral tube.

A remarkable feature of *Gladiolus liliaceus* is the color shift that occurs at sunset. As the tepals become more fully extended the normally beige, light brown or rusty colored tepals take on a yellowish background hue with the brown pigment changing to light blue-mauve. First reported by Henry Andrews in 1798, this color change has intrigued botanists ever since and is thought to be a direct adaptation to moth pollination (Goldblatt & Manning, 1998). A less pronounced color change at sunset



Figure 1. Comparison of the flowers of southern African *Gladiolus* pollinated by the satyrid butterfly *Aeropetes tulbaghia* (A, B) versus night-flying moths mostly of the families Noctuidae and Sphingidae (C–F), with longitudinal sections of flowers of some species. —A. *G. sempervirens* (sect. *Blandus*). —B. *G. nerineoides* (sect. *Linearifolius*). —C. *G. emiliae* (sect. *Linearifolius*). —D. *G. maculatus* (sect. *Homoglossum*). —E. *G. liliaceus* (sect. *Homoglossum*). —F. *G. guthriei* (sect. *Linearifolius*). Scale bar 10 mm.

also occurs in the related *G. hyalinus*, the tepals of which become paler and more translucent as daylight fades. The darkly mottled tepal coloration may be an adaptation for camouflage, rendering the flowers less visible to nectar or pollen thieves during the day. Johnson (1995) has suggested that the maroon pigmentation of the moth-pollinated flowers of the orchid *Monadenia ophrydea* Lindl. likewise represents camouflage. Moths are believed to locate flowers of this species solely by their scent.

Flowers pollinated by the *Aeropetes* butterfly are fairly large (Table 2), with the exception of *Gladiolus nerineoides*, and have a perianth tube mainly 30–45 mm long, and the dorsal tepal, usually slightly larger than the other five tepals, is mostly 35–65 mm long, thus somewhat longer to about twice as long as the tube. In *G. nerineoides* the somewhat smaller flowers have a floral tube 25–31 mm long and tepals 19–22 mm long, thus shorter than the tube. The perianth tube is more or less cylindric but slightly wider toward the apex, with the slender lower portion 1.5–2 mm in diameter, and fairly straight or gently curved. Flowers are zygomorphic (barely so in *G. nerineoides*) and the larger dorsal tepal either more or less erect, or lightly inclined (*G. cardinalis*), while the upper lateral tepals spread outward. The lower tepals are usually slightly smaller than the upper three and are held loosely together and directed forward. The style and stamens are unilateral and extend outward, and well exerted from the tube. Exceptional *G. nerineoides* has subequal tepals spreading more or less at right angles to the tube and the filaments are included, while the anthers may be partly exerted or entirely included.

Butterfly-pollinated flowers are shades of red, or exceptionally deep pink (*Gladiolus carmineus*), and the lower tepals are often streaked with white. In *G. saundersii* the lower tepals may be almost entirely white with irregular reddish speckling (Table 2). The flowers of all the butterfly-pollinated species lack detectable odor (Table 2). In the sense of Faegri and van der Pijl (1979), these are flag flowers but with a particularly elongated floral tube. The flowers of *G. insolens*, *G. nerineoides*, and *G. stokoei* are uniformly colored.

NECTAR ANALYSIS (TABLE 3)

Nectar produced by moth-pollinated species of *Gladiolus* is sucrose-dominant and ranges in concentration from a low of 19.5% (*G. emiliae*) to 36.4% (*G. tristis*) sucrose equivalents. Nectar quantities, measured from unbagged flowers, range from 2.2 to 12.4 μ l.

Nectar of butterfly flowers is markedly variable in character, and ranges from sucrose-dominant (*G. cardinalis*, *G. stefaniae*) to sucrose-rich (*G. cruentus*, *G. saundersii*) or hexose-rich (*G. insolens*, *G. nerineoides*) according to the definition of Baker and Baker (1983). These nectars also have relatively low concentrations, ranging from a low of 18–21% sucrose equivalents in *G. stefaniae* to 26.8% in one of two populations of *G. nerineoides* examined.

FRAGRANCE

Moth-pollinated species of *Gladiolus*, with one exception, produce strong, and often rich sweet odors (Table 4). In species of *Gladiolus* series *Tristis* fragrance is produced at nightfall and is weak or evidently absent during the day. Fragrances vary considerably as perceived by the human nose, though often appear to have a strong clove component and thus resemble the scent of stocks (*Matthiola*) or carnations (*Dianthus*). The odor produced by *G. albens* (sect. *Homoglossum*) is strikingly different, and is somewhat acrid and metallic, while the odor produced by *G. emiliae* (sect. *Linearifolius*) is fruity, with elements of coconut and pineapple. Scent production in *G. hyalinus* is evidently uncommon. In four populations we examined (Die Galg, Gydo Pass, Lion's Head, Nieuwoudtville), all in the west of its geographic range, flowers produced no detectable odor, but collection notes with some herbarium records from the eastern half of its range indicate the presence of a strong, sweet scent. Absence of odor is combined with mottled, brownish or purplish coloration in these populations.

Scent compounds mostly belong to different chemical classes of scents from those of bee-pollinated flowers (Goldblatt et al., 1998a), excepting for linalool, which is present in some bee-pollinated species, notably *G. alatus*, while benzyl acetate is also present in *G. jonquiliodoris*. Scent chemical profiles differ considerably among moth-pollinated species, even though linalool is a common component (Table 4) and is the predominant compound in *G. maculatus*. As in bee-pollinated species of the genus, numerous compounds combine to produce the characteristic scent of each species, and as many as 39 compounds were identified in *G. recurvus* (R. Kaiser, pers. comm.). Surprisingly, *G. acuminatus*, the only species of *Gladiolus* sect. *Hebea* examined, shares no compound with the moth flowers of species of section *Homoglossum* to which belong the other species analyzed. The spicy-clove type scents of *G. liliaceus* and *G. tristis* appear to derive from different compounds, eugenol in the former and eucalyptol in the latter.

Table 3. Available nectar characteristics of species of southern African *Gladiolus* pollinated by Lepidoptera. Nectar analyses were provided by B.-E. van Wyk, Rand Afrikaans University, Johannesburg, South Africa. Number of samples (n) is the same for volume and concentration columns. Data marked with an asterisk (*) are from Johnson and Bond (1994).

<i>Gladiolus</i> species	Nectar volume µl (n)	% Nectar concentration (±SD)	% Range of sugars			Sugar ratio S/F + G (n)
			Fructose	Glucose	Sucrose	
GLADIOLUS SECTION BLANDUS						
series <i>Blandus</i>						
<i>G. cardinalis</i>	*9.4 (12)	*24.8 (n/a)	9	16	75	3.0 (1)*
<i>G. carmineus</i>	1.7–4.8 (10)	26.1 (3.6)	18	30	52	1.08 (1)*
<i>G. insolens</i>	4.8–7.6 (3)	—	39	38	23	0.26 (1)
<i>G. stefaniae</i>	8.6–11.0 (2)	18–21	10–16	18–25	59–72	1.89 (2)
			*10	12	78	3.5 (1)
GLADIOLUS SECTION HEBEA						
series <i>Permeabilis</i>						
<i>G. robertsoniae</i>	3.8–4.5 (2)	—	—	—	—	—
GLADIOLUS SECTION LINEARIFOLIUS						
series <i>Linearifolius</i>						
<i>G. emiliae</i>	3.5–6.6 (3)	22.3 (1.5)	—	—	—	—
	5.7–6.4 (2)	19.5–20.5	8–15	5–9	76–87	4.4 (2)
<i>G. guthriei</i>	4.4–4.8 (5)	31.4 (3.0)	—	—	—	—
<i>G. nerineoides</i> Helderberg	1.8–4.2 (6)	26.8 (2.3)	12–26	23–36	37–63	1.85 (3)
Jonkershoek	2.9–4.6 (3)	23.7 (2.6)	28	46	26	0.35 (1)
GLADIOLUS SECTION HOMOGLOSSUM						
series <i>Gracilis</i>						
<i>G. albens</i>	3.8–5.6 (4)	27.0 (2.2)	—	—	—	—
<i>G. maculatus</i>	4.8–6.0 (2)	28–30	—	—	—	—
<i>G. recurvus</i>	4.1–13.7 (6)	31.9 (2.0)	6–7	11–14	80–82	4.3 (2)
series <i>Tristis</i>						
<i>G. hyalinus</i> Gydo Pass	2.2–4.7 (3)	35.8 (4.8)	2–3	7–8	90	9.0 (2)
Lion's Head	—	—	0–4	2–7	89–98	13.8 (3)
<i>G. liliaceus</i>	3.5–6.4 (5)	35.2 (1.2)	1	2–3	96–97	26.0 (3)
<i>G. longicollis</i>	2.7–3.7 (3)	24.7 (2.5)	6–27	9–23	50–85	2.2 (2)
subsp. <i>platypetalus</i>	5.1–7.9 (3)	28.3 (1.5)	6–11	6–11	78–88	4.3 (2)
<i>G. tristis</i>	8.5–12.4 (5)	36.4 (2.1)	—	—	—	—
GLADIOLUS SECTION OPHIOLYZA						
<i>G. cruentus</i>	4.8–5.7 (3)	20.7 (2.5)	26.5	39	35.5	0.55 (2)
<i>G. saundersii</i>	14.7–20.1 (5)	24.7	15	42	43	0.75 (1)

POLLINATION MECHANISMS AND POLLEN LOAD
ANALYSES

Moth pollination. Observations of pollinators on 7 species of the 11 putatively moth-pollinated southern African *Gladiolus* species showed all of them to be visited by night-flying moths (Table 5). Visitors included sphinx moths alone (*G. longicollis*), sphinx and noctuid moths (*G. emiliae*), or a range of small and larger moths including Noctuidae and other families. No other animals were noted visiting these species either during the day or at night excepting for a single male *Anthophora diversipes* (Apidae: Anthophorinae) captured while attempting to forage for nectar on the long-tubed flowers of *G. recurvus*. Its activities did result in contact of both anthers and stigmatic surfaces, and

Gladiolus pollen was recovered from its body. This bee should probably not be considered a regular pollinator of the species since the reward offered is not accessible to the bee, which has a tongue up to 12 mm long, while the tube of *G. recurvus* is at least 27 mm long.

Details of moth visits are limited because of the difficulty of observing their activity in the dark or under low intensity red light. Except for species of Sphingidae, moths settled on flowers, grasping the lower tepals, before inserting their probosces into the floral tube (Fig. 2). Too few visits were noted on any species for us to make observations of the duration of visits—we were determined first to capture visiting moths for identification of species and location of sites of pollen deposition. Captured

Table 4. Scent characteristics of selected species of southern African *Gladiolus* with flowers adapted for moth pollination (R. Kaiser, pers. comm.).

Scent composition (% constituents above 2%)														
Species	Scent description	Benzaldehyde	Benzyl alcohol	Benzyl acetate or benzoate	Eugenol	Linolool	Methyl benzoate	Eucalyptol	Phenylacet-aldehyde	(E)-ocimene	Epoxy-3,7-dimethyl 6-octadiene	(E)-ocimene epoxide	(E, E)-alpha farnesene and farnesol	(E)-cinnamic alcohol
<i>G. acuminatus</i>	floral	—	—	—	—	—	—	—	—	31.0	6.2	6.8	7.7	—
<i>G. liliaceus</i>	clove	68.0	—	—	14.1	—	8.1	—	4.0	—	—	—	—	—
<i>G. maculatus</i>	lily	—	—	—	—	91	—	—	—	—	—	—	—	—
<i>G. recurvus</i>	gardenia	—	12.6	6.9	—	69.5	—	—	—	—	—	—	—	5.8
<i>G. tristis</i>	clove	—	—	5.8	3	61.5	10.5	9.5	—	—	—	—	—	—

moths were found to carry *Gladiolus* pollen on the proboscis, but no pollen was recovered from moth bodies. After being netted and transferred to a killing bottle, moths lose many of their body scales and may have also lost any pollen they might have carried. Nevertheless, those moths with elongate probosces, including the sphingids *Hippotion* and *Agrius* and the noctuid *Cucullia*, must be regarded as legitimate pollinators of the species on which they were captured. Moths in the families Archiidae and Geometridae with shorter probosces, 10 mm long or less, that were also captured, cannot reach nectar in the floral tubes and must be regarded as accidental visitors, perhaps attracted by the strong floral odors.

Butterfly pollination. Five species (Table 5) were seen to be visited by *Aeropetes tulbaghia* and another four species (*Gladiolus carmineus*, *G. cruentus*, *G. insolens*, and *G. stokoei*) have similar flowers and are inferred to share this pollination strategy. This large butterfly has a wingspan of ca. 80 mm, a large body ca. 20 mm long, and a proboscis 28–34 mm long (Johnson & Bond, 1994; Johnson, 1994). *Aeropetes* was seen fluttering around flowers of *G. cardinalis*, *G. nerineoides*, *G. saundersii*, *G. sempervirens*, and *G. stefaniae* (spanning three sections, *Blandus*, *Linearifolius*, and *Ophiolyza*, Table 5), sometimes settling for 25 to 130 seconds, and then to fly to other flowers of the same species. As Johnson and Bond (1994) have noted, the behavior of butterflies included inspection visits when individuals flutter above flowers without settling or feeding. During such visits, the ventral part of the thorax, abdomen, and wings may brush against the well-exserted anthers or stigmatic surfaces of species like *G. cardinalis*, *G. sempervirens*, and *G. stefaniae* (sect. *Blandus*). Pollen and stigmatic surfaces of *G. nerineoides* (sect. *Linearifolius*) and *G. saundersii* (sect. *Ophiolyza*) are only contacted during feeding visits when insects settle, grasping the tepals and inserting their proboscis into the perianth tube. In *G. nerineoides* pollen is deposited only on the upper proboscis because the anthers are not or are only shortly exerted from the floral tube. Pollen is deposited on the head, antennae, and dorsal part of the thorax of *G. saundersii* because the hooded dorsal tepal, under which the anthers lie, forces a feeding insect to orient its body nearly vertically as it grasps the upper lateral or lower tepals. No other animals were ever seen visiting those *Gladiolus* species that attracted *Aeropetes*, and this single insect must be assumed to be their sole pollinator.

The innate attraction that *Aeropetes* exhibits for red flowers, particularly those of large size, or col-

Table 5. Length of perianth tube of *Gladiolus* species (from Goldblatt & Manning, 1998, representing the ranges for the species, measured from all known herbarium collections) and mouthparts of captured moths. Few butterflies were captured because their identity is not in question; the few individuals captured were released after sites of pollen deposition were noted because *Aeropetes* is a legally protected species. Family affiliations: Archiidae: *Hypagoptera*; Geometridae: *Macaria*; Noctuidae: *Cucullia*, *Syngrapha*, *Tychopmoptes*; Satyridae: *Aeropetes*; Sphingidae: *Agrius*, *Hippotion*. Note: the “spurge hawkmoth” reported on *G. longicollis* by J. M. Wood (in Scott Elliot, 1891) may also have been *A. convoluli*.

Plant species	Perianth tube (mm)	Lepidopteran species (n)	Mouthpart (mm)	Position of pollen on insect body
GLADIOLUS SECTION OPHIOLYZA				
<i>G. saundersii</i>	33–37	<i>Aeropetes tulbaghia</i>	not measured	frons, dorsal thorax
GLADIOLUS SECTION BLANDUS				
<i>G. cardinalis</i>	30–45	<i>Aeropetes tulbaghia</i>	not captured	not available
<i>G. sempervirens</i>	30–45	<i>Aeropetes tulbaghia</i>	not captured	not available
<i>G. stefaniae</i>	44–55	<i>Aeropetes tulbaghia</i>	not captured	not available
GLADIOLUS SECTION LINEARIFOLIUS				
<i>G. guthriei</i>	16–18	<i>Cucullia inaequalis</i> (1)	ca. 16	distal 12 mm of proboscis
<i>G. emiliae</i>	32–45	<i>Hippotion celerio</i> (1)	ca. 30	distal 25 mm of proboscis
		<i>Cucullia inaequalis</i> (1)	ca. 28	distal 25 mm of proboscis
		<i>Cucullia extricata</i> (1)	ca. 20	distal 15 mm of proboscis
		<i>Macaria simplicilinea</i> (1)	<10	no pollen
		<i>Hypagoptera</i> sp. (1)	<10	no pollen
<i>G. nerineoides</i>	16–18	<i>Aeropetes tulbaghia</i>	not captured	not available
GLADIOLUS SECTION HOMOGLOSSUM				
<i>G. liliaceus</i>	40–45	<i>Cucullia extricata</i> (1)	ca. 28	distal 25 mm of proboscis
<i>G. longicollis</i>	85–110	<i>Agrius convolvuli</i> (1)	110–115	25 mm from base of proboscis
<i>G. maculatus</i>	23–35	<i>Cucullia terensis</i> (1)	25–30	distal 25 mm of proboscis
		<i>Cucullia</i> sp. (1)	15–20	from base of proboscis
<i>G. recurvus</i>	27–36	<i>Hippotion eson</i> (1)	ca. 40	distal 25 mm of proboscis
<i>G. tristis</i>	40–60	<i>Syngrapha circumflexa</i> (3)	16–17	dorsal thorax and proboscis
		<i>Tychopmoptes inferior</i> (1)	ca. 10	no pollen

lectively making a prominent display, as in *G. nerineoides*, is well known and has been documented elsewhere (Johnson & Bond, 1994; Goldblatt et al., 1999). The wavelength range of red color exhibited by *Aeropetes* flowers is virtually the same as that in red-flowered species pollinated by sunbirds. There is no evidence of effective pollination of the butterfly-pollinated species of *Gladiolus* by sunbirds as there is, for example, in red, tubular-flowered species of *Tritoniopsis* and *Watsonia* (Iridaceae), *Kniphofia* (Asphodelaceae), and *Erica* (Ericaceae), which may use both sunbirds and *Aeropetes* equally as pollinators (Johnson & Bond, 1994; Goldblatt et al., 1999). Possibly the inclined, and somewhat flaccid stem and difficulty in reaching the perianth tube because of the large, widespread tepals discourage sunbird visits. In connection with *Aeropetes* pollination, the record of this butterfly on *G. cruentus* (Johnson & Bond, 1994) must be mentioned. From the locality (J. J. Vlok, pers. comm.) this is clearly for the high-altitude Drakensberg species, *G. flanaganii*, and probably reflects the innate attraction to red color by *Aeropetes*. The very fleshy,

stiff cupped tepals, sturdy flowering stem, and hooded dorsal tepal of this species (Goldblatt & Manning, 1998) are suited to sunbird pollination but seem unsuited to butterfly pollination, as does the nectar, which has a concentration of 35% sucrose equivalents (Goldblatt et al., 1999). The report of the *Nectarinia famosa* visiting *G. flanaganii* is consistent with the floral adaptations of the species, and *Aeropetes* may simply have been a casual visitor. *Gladiolus cruentus* is restricted to lower elevations inland from the central KwaZulu–Natal coast (Goldblatt & Manning, 1998) where *Aeropetes* is rare or normally absent, and this red-flowered species may be pollinated by alternative large butterflies, such as *Papilio demodocus* and *P. nereis*, as has been found for lower-elevation populations of another red-flowered species of Iridaceae, *Hesperantha coccinea* (Backh. & Harv.) Goldblatt & J. C. Manning (Goldblatt et al., in press).

DISCUSSION

Although aspects of pollination of *Gladiolus* species by moths and the satyrid butterfly *Aeropetes*

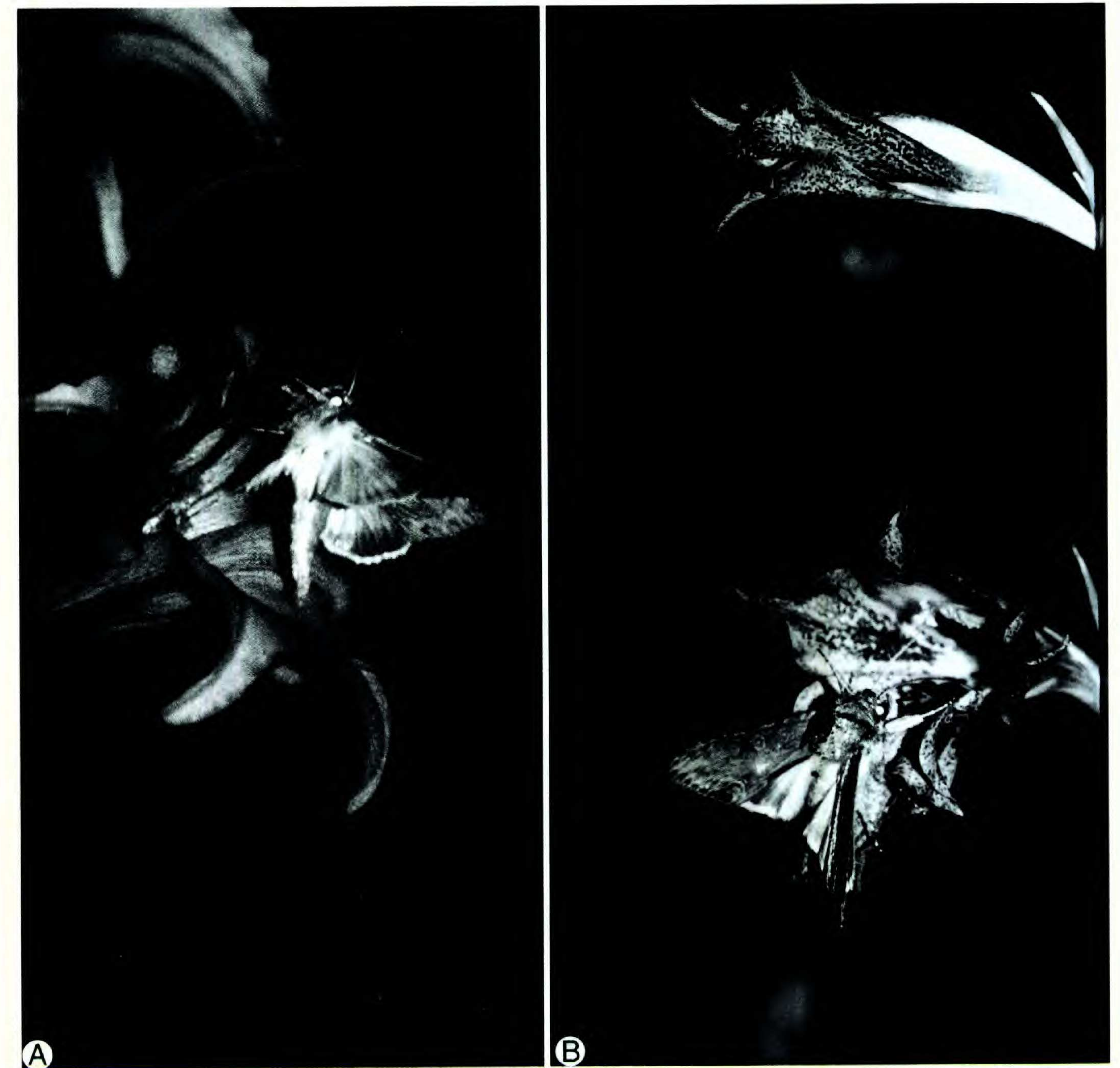


Figure 2. Noctuid moths photographed while visiting flowers of *Gladiolus*.—A. *Cucullia extricata* on *G. liliaceus*.—B. *Cucullia* cf. *terensis* on *G. maculatus* (this particular moth was not captured).

have already been documented (Johnson & Bond, 1994) or discussed in more general terms (Goldblatt & Manning, 1998; Goldblatt et al., 2001), these two quite different pollination systems are more fully documented here. Moreover, comparison of moth- or butterfly-pollinated species with their closest relatives with different pollination systems makes it possible for us to draw reasonable conclusions about the evolution of both pollination systems in the genus, for which a hypothetical phylogeny already exists (Goldblatt & Manning, 1998). Moth pollination (Table 6) has evolved in three sec-

Table 6. Analysis of the frequency and taxonomic distribution of night-flying moth and butterfly pollination systems in southern African species of *Gladiolus*. Numbers in parentheses in columns 2 to 5 represent number of species inferred to have the pollination system.

Pollination system	Section					Total species confirmed	Taxonomic sections (series)	Total confirmed and inferred
	<i>Ophiolyza</i>	<i>Blandus</i>	<i>Linearifolius</i>	<i>Hebea</i>	<i>Homoglossum</i>			
Moth	0	0	2 (0)	0 (2)	5 (2)	7	3 (5)	11 (7%)
Large butterfly	1 (1)	3 (2)	1 (1)	0	0	5	3 (3)	9 (5%)

tions of *Gladiolus*, *Hebea*, *Homoglossum*, and *Linearifolius*, while butterfly pollination evolved in *Gladiolus* sects. *Blandus*, *Ophiolyza*, and *Linearifolius*. Within section *Hebea* the two moth-pollinated species, *G. acuminatus* and *G. robertsoniae*, have such different flowers that we do not believe they are immediately related. More likely they evolved from different, bee-pollinated ancestors. Similarly, the moth-pollinated species of section *Homoglossum* belong in different series (Tables 1, 2). In addition, in series *Gracilis* both floral and leaf morphological differences between *G. recurvus*, which has apomorphic leaves with winged margins, like those of *G. gracilis* Jacq., and *G. albens* and *G. maculatus*, which have leathery leaves without prominent margins or midribs, suggest that *G. recurvus* is not immediately related to the latter species pair. Lastly, in *Gladiolus* sect. *Linearifolius*, the only section with both moth- and butterfly-pollinated species, species with the two pollination systems fall in different lineages (Goldblatt & Manning, 1998).

These considerations led to the hypothesis that moth pollination arose six times within the southern African species of *Gladiolus* while butterfly pollination arose at least three times (Table 6). Comparison of the pollination systems of immediate ancestors of moth- or butterfly-pollinated species makes it likely that moth pollination arose from bee-pollinated ancestors in *Gladiolus* sects. *Hebea* and *Linearifolius*, and in series *Gracilis* of section *Homoglossum*. The sister clade of series *Tristis*, series *Homoglossum*, comprises only bird-pollinated species, but we cannot say whether the predominantly moth-pollinated series *Tristis* evolved from bird-pollinated ancestors or the putative common ancestor of the two series, series *Teretifolius*, which comprises mostly bee-pollinated species (Goldblatt & Manning, 1998; Goldblatt et al., 1998a). In tropical Africa, *Gladiolus* sect. *Acidanthera*, with seven species, also shows all of the classic adaptations for moth pollination. The perianth is white and the perianth tube 60–150 mm long depending on the species (Goldblatt, 1996), and flowers produce a strong odor that, at least for the widely cultivated *G. murielae* Kelway (= *G. abyssinicus* (Brongn. ex Lemaire) Goldblatt), is strongest at night (unpublished obs.). The flowers of two species of the section, *G. aequinoctialis* Herbert and *G. murielae*, are unusual for moth pollination in having dark blotches on the lower tepals, and the entire section *Acidanthera* has an unusual specialization, anthers with stiff, acute apical appendages (Goldblatt, 1996). Because of the length of the perianth tube in species of section *Acidanthera*, mostly 90–140

mm long, pollination by sphinx moths (Sphingidae), which have probosces of comparable length, is inferred but there are no pollination studies for the *Gladiolus* species of tropical Africa.

One notable aspect of the evolution of moth pollination in the three southern African sections of the genus in which it occurs is that these sections (*Gladiolus* sects. *Hebea*, *Homoglossum*, and *Linearifolius*) are the only ones in which floral fragrance is well developed (and is associated primarily with bee pollination). As we have noted elsewhere (Goldblatt et al., 1998a), floral fragrance is common in the southern African species of the winter-rainfall zone where sections *Hebea* and *Homoglossum* are best represented. It is also present in a few summer-rainfall zone species of the above sections that have their closest allies in the winter-rainfall zone. The scarcity of moth-pollinated species in summer-rainfall parts of southern Africa may be the result of the absence of scent in sections of the genus centered there. In tropical Africa scent in moth-pollinated species of section *Acidanthera* appears to have evolved independently within the section (the section as currently defined does not occur in southern Africa). Floral odor in *Gladiolus* species (Goldblatt et al., 1998a) is the result of the combination of many substances and in moth flowers perceived odor represents different mixes and proportions of these same series of compounds.

Apart from fragrance, moth flowers differ from their bee-pollinated ancestors in perianth tube length, color, and size of the tepals. Large size is probably important for display purposes at night as is the pale coloration. An unusual aspect of moth-pollinated flowers in *Gladiolus* is the frequency of dull patterning or mottling on a pale background, most notable in *G. hyalinus*, *G. liliaceus*, *G. maculatus*, and even some forms of *G. longicollis* (all sect. *Homoglossum*) and in *G. emiliae* and *G. guthriei* (sect. *Linearifolius*). Dark mottling is less pronounced in *G. hyalinus* and *G. liliaceus* at night, as the pigments either change in intensity or color or both. The mottling may be camouflage, protecting flowers from predation during the day, as Johnson (1994) has suggested for the maroon-flowered orchid, *Monadenia ophyridea*, which is presumably located by its settling moth pollinators by scent alone.

Butterfly-pollinated flowers in *Gladiolus* sect. *Blandus* appear to have evolved from ancestors adapted for pollination by long-proboscid flies, the predominant pollination system in the section, and the one present in species most closely allied to the clade of butterfly-pollinated species in the section (Goldblatt & Manning, 1998, 1999). Long-probos-

cid fly flowers have similarly long floral tubes and differ most significantly in floral pigmentation, cream or pink with red nectar guides often outlining a white zone. In *Gladiolus* sect. *Ophiolyza* the ancestors of the two butterfly-pollinated species have flowers adapted for pollination by sunbirds (the *G. dalenii* complex) (Goldblatt et al., 1999). In series *Linearifolius* butterfly-pollinated *G. nerineoides* and *G. stokoei* (putatively butterfly pollinated) fall within a clade with the bee-pollinated *G. brevifolius* and long-proboscid fly pollinated *G. monticola* (Goldblatt & Manning, 1998, 1999).

At first it might appear that a simple shift from ancestral pink or cream flowers to red ones may effect a shift to butterfly pollination. In *Gladiolus* increase in flower size appears equally important. Moreover, other more subtle changes suggest that more complex genetic adaptations are necessary, including a shift in the position of the anthers and stigmatic surfaces, either prominently exerted or included in the floral tube, and a shift in flowering time (butterflies are on the wing only in late summer). In the winter-rainfall zone a whole series of vegetative and physiological adaptations would also be required to produce flowers in an area of summer heat, drought, and low atmospheric humidity, a time that necessitates the separation of vegetative growth from the flowering phase of the life cycle.

Johnson and Bond (1994) have pointed out a feature of butterfly pollination in the southern African winter-rainfall zone that relates directly to this question. This is that so many butterfly-pollinated species of the area are rare, or are highly local endemics. We suspect this has nothing to do with the intrinsic effects of having a specialist pollination system and is correlated instead with the fact that species flowering in the late summer and autumn in an area of mostly winter precipitation require highly specialized habitats that are by their very nature highly local. Thus, *G. cardinalis* survives summer drought by growing along watercourses in cool mountain habitats, often in the spray of perennial waterfalls. *Gladiolus sempervirens*, *G. stefaniae*, and *G. stokoei* also grow in montane habitats and are restricted to slopes with poor drainage where the summer southeast winds bring moisture in the form of fog that keeps the ground moist at this otherwise dry season. *Gladiolus nerineoides* is restricted to shaded south-facing cliffs where moisture percolating from the slopes above keeps their substrate relatively damp. Even in these specialized habitats vegetative specialization makes survival for these summer-flowering species possible. *Gladiolus nerineoides*, *G. stefaniae*, and *G. stokoei* produce foliage leaves in the winter, after the wet

season has begun. Their flowering stems bear short, often entirely sheathing, and sometimes dry leaves while the foliage leaves are dry and often lost by the time flowers are produced.

An aspect of evolution of specialized pollination systems is a change in the nature of the reward. Bee-pollinated species of *Gladiolus* mostly produce sucrose-rich or sucrose-dominant nectar of relatively high concentration (Goldblatt et al., 1998a). The pattern in moth-pollinated flowers is similar, although sugar concentration is often higher. This pattern is one that is common in the Iridaceae. In moth-pollinated species of *Hesperantha* (Goldblatt et al., in press) nectar sugars usually have concentrations of 45–50% sucrose equivalents. The relatively high volume of nectar in most moth flowers, especially compared to their presumed bee-pollinated ancestors, may relate to the higher nutrient needs of active, fast moving moths with relatively large bodies, especially in the case of Sphingidae, which hover while feeding.

Unlike nectar of moth flowers, the nectar of butterfly-pollinated *Gladiolus* species shows a lower sugar concentration than their presumed long-proboscid fly or bee-pollinated ancestors (Goldblatt et al., 1998a; Goldblatt & Manning, 1999), but a relatively high volume. Exactly why the lower nectar sugar concentration is characteristic of large butterfly pollination is uncertain. The higher viscosity of concentrated nectar may make it difficult for an insect to draw through the proboscis, but this is evidently not a consideration for small or large moths, which have similarly constructed and sometimes equally long and narrow mouthparts. Another feature of butterfly nectars in *Gladiolus* is the shift to higher proportions of hexose sugars. While most species of section *Blandus* have the sucrose-dominant type nectar found in related long-proboscid fly pollinated species (Goldblatt & Manning, 1999), *G. insolens* has hexose-rich nectar. A similar but less pronounced shift is evident in the two butterfly-pollinated species of section *Ophiolyza*, which have sucrose-rich nectar with nearly equal proportions of sucrose and hexose sugars, and in *G. nerineoides*, which shows the same shift, while related species have nectar with higher proportions of sucrose (Goldblatt & Manning, 1999; Goldblatt et al., 1999). Such changes in nectar sugar chemistry are rare in the Iridaceae, especially in the subfamily Crocoideae (syn. Ixioideae) to which *Gladiolus* belongs, and are presumably pollinator driven, reflecting a preference of *Aeropetes* for nectar with elevated levels of hexose sugars, rather than a random change.

Shifts in pollinators are common within the large

er genera of the African Iridaceae and are an important factor in the radiation of the family. Elsewhere we have speculated that as many as 32 shifts in pollination system have occurred just in *Gladiolus* in southern Africa (Goldblatt et al., 2001). This represents an average of one shift for every five species. The convergent evolution of moth pollination at least six times and butterfly pollination three times reflects the evolutionarily labile floral and vegetative morphology of this highly successful genus. As outlined above, shifts that may appear quite simple, appearing to represent changes mainly in pigmentation and floral tube length are, in fact, complex and represent a closely integrated series of adaptations that include, in addition to floral patterning, shifts in fragrance characteristics, timing of the opening and closing of the tepals and sometimes scent release, nectar physiology, seasonal phenology, and associated vegetative adaptations for those species of the southern African winter-rainfall zone that flower in summer. The developmental complexity associated with these shifts makes their high frequency seem even more impressive.

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